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Research Article
Effects of Supplementation of Different Sources of Tannins on Nutrient Digestibility, Methane Production and Daily Weight Gain of Beef Cattle Fed on Ammoniated Oil Palm Frond Based Diet

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Abstract
Background and Objective: Tannins have the ability to reduce methane production in ruminants, thereby increasing the efficiency of the utilization of energy and optimizing animal body weight gain. This study was conducted in order to determine the effect of supplementation of tannins from two different sources of ammoniated oil palm frond in diets based on ammoniated oil palm as a source of roughage, on the feed intake, digestibility and daily weight gain of beef cattle. The source of the tannin is gambier leaf waste (GLW) from Lima Puluh Kota and Pesisir Selatan, two districts in West Sumatra province. **Materials and Methods:** The study was designed using Latin Square Design (LSD). Treatment A, the control, was a complete cattle feed consisting of oil palm frond pre-treated with 6% urea+concentrate. Treatment B was Diet A+10% GLW Painan and treatment C was diet A+15% GLW Payakumbuh. Each treatment had a roughage to concentrate ratio of 50:50. Parameters measured were feed intake, nutrient digestibility, body weight gain and methane production. **Results:** Results showed that treatments had no significant (p>0.05) effects on intakes of dry matter and organic matter, but did have a significant effect (p<0.05) on nutrient digestibility, average daily gain and methane production. Digestibility of dry matter increased from 59.95% (treatment A) to 62.02 and 63.52% with treatments C and B, respectively. Methane production decreased from 2.48 MJ/day (treatment A) to 1.28 MJ/day and 1.26 MJ/day with treatments B and C, respectively and daily weight gain increased from 0.65-0.90 and 0.95 kg/day. **Conclusion:** The results showed that the supplementation of GLW increased nutrient digestibility and daily weight gain and reduced methane production. There was no significant difference between sources of GLW.

Key words: Ammoniated oil palm frond, gambier leaf waste, methane production, nutrient digestibility, daily weight gain

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The farming of ruminants is the source of 12-41% of atmospheric methane emissions from all agricultural sources[1]. This contributes to the build-up of green house gases in the atmosphere and increases global warming[2]. This gambier leaf waste (GLW) from Lima Puluh Kota and Pesisir Selatan is source of efficient use of energy from feed. Approximately 6-10% of the gross available energy from feed consumed by ruminants is lost as methane[3].

Several attempts have been made to reduce methane emissions, including supplementation with feed concentrates[4], lipids[5], organic acids[6,7], essential oils[8] and probiotics and prebiotics[9,5]. These have been tested *in vitro* and *in vivo*. Antibiotic compounds such as monensin and lasalocid have also been used to reduce methane production[10]. However, use of antibiotics has been banned in the European Union since 2006 and other countries outside of Europe are in the process of considering banning antibiotics. This has resulted in increased efforts by researchers to find naturally occurring compounds in plants that can be used as feed additives to increase cattle production[11-13] and reduce methane emissions[14]. Tannins, also called polyphenols, are a class of naturally occurring compounds from plants that could potentially reduce methane emissions[15]. Tannins have the ability to act as anti-methanogenic agents in the rumen with effectiveness dependent upon the type and dosage of tannin. Low molecular weight tannins are more effective inhibitors of methanogenic bacteria than higher molecular weight tannins, as they form stronger bonds with microbial enzymes. Higher molecular weight tannins are unable to penetrate the microbial protein cell wall and therefore, have a lower toxicological effect on methanogenic bacteria[16]. Ningrat *et al.*[17], conducted *in vitro* tests on feed supplementation with GLW from two different locations and found that diets containing 10% waste from Painan and 15% waste from Payakumbuh reduced methane emissions the most. The difference in the optimal percentage of GLW from each source was due to their different tannin levels.

This study was conducted *in vivo* using these two different sources of GLW with an ammoniated oil palm frond diet to measure the effects on methane emission and digestibility.

MATERIALS AND METHODS

The experiment was conducted from December, 2016 to February, 2017. This study used Simental cattle that were available in farm of Animal Science Faculty, Andalas University.

Table 1: Composition and nutritional content of experimental diet (% DM)

	Diet (% DM)		
Items	A	B	C
Ammoniated palm frond	50.0	45.0	42.5
Rice brand	5.00	4.50	4.25
Palm kernel cake	33.50	33.15	28.575
Corn	10.00	9.00	8.50
Mineral	1.00	0.90	0.85
Salt	0.50	0.45	0.425
Gambier leaf waste	-	10.00	15.00
Total	100.0	100.0	100.0
Nutrient contents			
Crude protein	12.98	12.73	12.72
Fat	4.25	4.13	3.99
TDN	63.28	62.77	62.30
NDF	56.04	57.19	55.86
ADF	42.03	44.43	42.18

DM: Dry matter, TDN: Total digestible nutrient, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

The experimental design used was a 3×3 Latin square. Each experimental period lasted 21 days, with 14 days for adaptation and 7 days for sampling. The animals were weighed at the beginning and at the end of each experimental period. Materials included ammoniated oil palm fronds, palm kernel cake, maize bran supplemented with minerals, GLW and three 18-20 months old Simental cattle with body weights between 179 and 190 kg. Feed composition was shown in Table 1.

The three treatments trialled were:

A = Control (palm kernel cake+concentrate)
B = A+10% Painan GLW (1.56% tannin)
C = A+15% Payakumbuh GLW (1.23% tannin)

Research implementation: Feed was given twice a day between 08.00 and 14.00 based on dry weight requirements (3% of body weight). Feed residue, feces and urine were collected. The uneaten feed was weighed daily to determine consumption rates. Body weight was also measured at the beginning and end of the period to determine weight gain. Feces were collected every 5 days and weighed. Efforts were made to keep the feces and urine separate. Feces were homogenized with a mixer and a 100 g sample was collected for proximate analysis. This sample was wrapped in a measured weight of aluminum foil, dried in a 60°C oven for 24 h until constant weight, ground to powder and then analyzed to determine the digestion of the elements in the feed. The collected urine was stored in glass containers with 10% H_2SO_4 for allantoin analysis. The resulting methane was measured using the equation[18]:

Methane (MJ/day) = 1,62 dCP-0,38d Cfat+3,78 dCF+149 dNFE+1142 kg/day

Where:

dCP = Digestible crude protein
dCfat = Digestible crude fat
dCF = Digestible crude fiber
dNFE = Digestible nitrogen free extract

Statistical analysis: A one-way analysis of variance was used to test for correlations between the trial parameters. Any correlation was tested further for statistical significance using the Tukey test[19]. A value of $p<0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Digestibility: The digestibility of the components of the feed used in this experiment was shown in Table 2. Analysis of variance indicated that a treatment has a statistically significant effect ($p>0.05$) on the digestibility of all components of the feed except hemicellulose. A subsequent Tukey test indicated that treatment A gives statistically significantly different results than treatment B or C, but there was non significant difference between these last two treatments. Addition of gambier leaf waste increases digestibility over the control. This was because of the tannin content in the gambier leaf waste. Tannin can disrupt protozoa, producing a conducive condition for bacteria to thrive[20]. Increased numbers of bacteria result in the production of higher levels of enzymes that assist the digestion of components in the feed. This was in agreement with previous study by Ningrat *et al.*[17], which found that tannins functioned as defaunation agents capable of reducing the protozoa population by almost 60%. McLeod[21] stated that tannins react with the protozoan cell wall disrupting membrane permeability, hence destroying protozoa. When fiber content is high, protozoa consume rumen bacteria, reducing their number and hampering food digestion. By reducing the protozoa population, the bacteria population is increased as is seen in the higher allantoin levels in the urine of the animals given gambier leaf waste. These allantoin levels in the urine were proportional to microbial protein synthesis. The higher allantoin levels in the urine, the higher the microbial protein synthesis. Microbial protein that enters the duodenum can be measured via urine allantoin levels, as they are the final product of the metabolism of purine, which is the most accurate measure of microbial protein synthesis[22].

Tannin in ruminant feed has advantages and disadvantages. Epidemiological studies indicated that 2-4% tannin in feed can prevent bloat while lower levels can impact ruminal fermentation and microbial protein synthesis[23].

Table 2: Gambir leaf waste (GLW) supplementation on the digestibility of the components of the feed and allantoin content in urine

	Treatments			
Parameters (%)	A	B	C	SE
DM digestibility	59.95^b	63.52^a	62.02^a	0.37
OM digestibility	65.98^b	70.45^a	70.52^a	0.29
CP digestibility	73.64	77.64	77.41	0.46
ADF digestibility	45.10^b	51.14^a	50.40^a	0.85
NDF digestibility	49.06^b	54.31^a	53.18^a	0.86
Cellulolytic digestibility	48.83^b	55.25^a	54.18^a	0.86
Hemicellulolytic digestibility	60.93	63.83	61.39	0.53
Allantoin urine (L/day)	197.04	227.59	204.383	25.5

^{a,b,c}Indicate statistically significant different values ($p<0.05$). A: Control (palm kernel cake+concentrate), B: A+10% Painan GLW (1.56% tannin), C: A+15% Payakumbuh GLW (1.23% tannin), DM: Dry matter, TDN: Total digestible nutrient, NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Table 3: Gambir leaf waste (GLW) supplement treatment on intake of feed nutrients, methane production and weight gain of the cattle

	Treatments			
Parameter	A	B	C	SE
DM Intake (kg/day)	4.62^a	4.50^a	4.47^a	0.04
OM Intake (kg/day)	4.06^a	3.95^a	3.93^a	0.03
CP Intake (kg/day)	0.60^a	0.58^a	0.58^a	0.01
ADG (kg/day)	0.65^a	0.90^b	0.92^b	0.014
Methane (MJ/day)	2.48^a	1.28^b	1.26^b	0.02

^{a,b,c}Indicate statistically significantly different effects ($p<0.05$). DM: Dry matter, OM: Organic matter, CP: Crude protein, ADG: Average daily gain

However, levels of tannins higher than 5% inhibit bacterial digestion and reduce the effectiveness of the rumination processes, particularly levels of consumption and digestibility of nutrients[24]. Apart from preventing bloat, another positive effect of the addition of tannin to feed is that it provides a protein bypass for organs in the digestive system after the rumen. This was because of the complex links between the tannins and protein that make it difficult for the protein to be degraded in the rumen, but it can then be digested further down the digestive system[11]. As seen in Table 2, this results in the higher digestibility of protein that occurred with GLW treatments.

Table 2 also indicated that there was no significant difference between the two sources or levels of tannin in the diet on the digestibility of protein. Different tannins have different levels of reactivity depending on the chemical structure and average molecular weight, which, if high, can make it difficult to degrade the protein[25].

Feed nutrient consumption, methane production and body weight gain: Nutrient consumption is closely tied to the energy supplied to the animal's system. The quality of the feed consumed must fulfill the nutritional requirements for meat production and the energy needed for the animal's activities. The average nutrient consumption for the cattle was shown in Table 3.

Analysis of variance indicated that treatment has no statistically significant effect ($p>0.05$) on feed consumption. Generally, as reported by Kumar and Vaithiyanathan[26], the addition of tannins to cattle feed tends to reduce feed consumption. Makkar *et al.*[27] stated that this reduction can have three causes: The feed is less palatable because of the astringency resulting from tannin-salivary protein bonds, bloating due to decrease in dry matter (DM) digestibility and the binding of tannin to the small intestine resulting in a hormonal response. According to Makkar[28], a decrease in animal feed intake has been observed only when the inclusion of tannin in diets was $\geq$3% of DM. In this study, the level of tannin inclusion was 1.5-1.9% of diet DM, hence, it would not be expected to affect DM intake (Table 3). These results were in accordance with those reported by Alves *et al.*[29], who found no influence of tannin inclusion in diets on DM intake.

Addition of GLW resulted in a small decrease in feed consumption was shown in Table 3. This reduction could be, if not from the bitter taste it gives the food, because the energy requirements of the cattle were being fulfilled with less feed, as they will not eat more than they require for their energy needs[30]. Addition of GLW as a tannin source reduces the bacteria population[16], thereby reducing methane production[31,17] and indicating that more of the energy in the feed was being utilized by the animal rather than being excreted as methane.

Methane gas production from ruminant enteric digestion, apart from contributing to global warming, indicated a loss of energy and lower efficiency in feed use, which represents an economic cost to the farmer. This research has shown that methane gas production was significantly reduced by these treatments. The GLW in treatments B and C resulted in lowered methane production as compared to the control diet. This indicated that the tannin content in the GLW was effective in reducing methane by reducing protozoa populations[20] and has a toxic effect on methanogenic bacteria[32].

As shown in Table 2, the increase in cattle body weight indicated that there is a statistically significant greater body weight gain in cattle feed with GLW compared to the control. This was a further indication of improved digestibility of the feed, presumably due to the tannin content that reduced the protozoa and methanogenic bacteria populations[33,32] and thereby resulted in more of the nutritional content of the feed being available for animal growth. Another factor could be that the tannin from the GLW results in less protein being degraded in the rumen, hence being available to be absorbed as amino acids further down the digestive system[25,34].

CONCLUSION

The results showed that addition of gambier leaf waste increases digestibility over the control; there was no significant difference between the two sources or levels of tannin in the diet on the digestibility of protein and there is no influence of tannin inclusion in the diets on DM intake. Inclusion of gambier leaf waste slightly reduced feed consumption and reduced methane production as compared to the control diet, however, higher body weight gain in cattle fed gambier leaf waste was comparable to the control.

SIGNIFICANCE STATEMENT

This study discovers the use of local feed ingredients containing tannins (gambier leaf waste) from two different districts in West Sumatra province increases bioprocess in the rumen and hence improve livestock productivity. The usage of ammoniated oil palm frond in the rumen improved through a combination of fortification technology using tannin to control microbial growth, so it can be beneficial as feed supplement in livestock rations. This study will help the researchers to produce feed supplement based on agricultural waste to get the feed affordable by the farmers. Thus, new opportunities to produce feed supplement from gambier leaf waste in combination with ammoniated oil palm frond and possibly other combinations.

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